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Intranasal Immunization with CpG Oligodeoxynucleotides as an Adjuvant Dramatically Increases IgA and Protection Against Herpes Simplex Virus-2 in the Genital Tract

W. Scott Gallichan,* Robert N. Woolstencroft,* Tina Guarasci, † Michael J. McCluskie, ‡ Heather L. Davis, ‡§ and Kenneth L. Rosenthal 2*†

Development of vaccines capable of preventing the transmission or limiting the severity of sexually transmitted viruses, such as HSV and HIV, will likely be dependent on the induction of potent long-lasting mucosal immune responses in the genital tract. Recently, synthetic oligodeoxynucleotides (ODN) containing immunostimulatory CpG motifs were shown to serve as potent adjuvants for the induction of mucosal immune responses. Here, we show that intranasal immunization with CpG ODN, plus recombinant glycoprotein B (rgB) of HSV-1, results in significantly elevated levels of specific anti-gB IgA Abs in vaginal washes that remained high throughout the estrous cycle. Additionally, dramatically elevated numbers of specific IgA Ab-secreting cells were present and persisted in the genital tract in response to intravaginal (IVAG) HSV-2 challenge. HSV-2-specific CTL were observed at moderate levels in the spleens of CpG or non-CpG ODN-immunized mice. In contrast, strong CTL responses were observed locally in the genital tissues of both groups following IVAG HSV-2 challenge. Interestingly, mice immunized intranasally with rgB plus CpG ODN, but not non-CpG ODN, were significantly protected following IVAG HSV-2 challenge. Measurement of virus in protected CpG-immunized mice revealed a log lower level of replication within the first few days after infection. In conclusion, these results indicate that intranasal immunization with CpG ODN plus protein mediates immunity in the female genital tract capable of protecting against a sexually transmitted pathogen.

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3 Abbreviations used in this paper: ODN, oligodeoxynucleotide(s); non-CpG ODN, ODN that do not contain CpG motifs; AdgB, recombinant human adenovirus type 5 expressing HSV glycoprotein B; ASC, Ab-secreting cell(s); ILN, iliac lymph node(s); i.n., intranasal(ly); gB, glycoprotein B; rgB, recombinant HSV-1 gB; IVAG, intravaginal(ly); ELISPOT, enzyme-linked immunospot.
female genital tract and compared with immunization with non-CpG ODN, rgB alone, or recombinant human adenosine type 5 expressing HSV gB (AdgB).

Materials and Methods

Animals

Inbred, female C57BL/6 mice (Charles River Canada, St. Constant, Quebec, Canada), 6–8 wk old, were used for these studies. Mouse colonies were maintained on a 12-h light/dark cycle.

Vaccine preparations and cell culture

Vero, 293, MC57, and LTA cells were grown in α-MEM (Life Technologies, Burlington, Ontario, Canada) supplemented with 10% FCS (Life Technologies) and 1% penicillin-streptomycin and 1-glutamine (Life Technologies). The production of AdgB has been previously described (28) and used as a mucosal vaccine against herpes infections (29–34). Mice were immunized with 2–5 × 10^9 PFU of AdgB. HSV-2 strain 333 was propagated and titrated on Vero cells. The CpG ODN (5′-TCCATGACGTTCCGACGT3′) and non-CpG control ODN (5′-TCCAGACTTCTCTCAGGT3′) (Coley Pharmaceutical Group, Wellwies, MA) were used at 10 μg/immunization. Both ODN had a nuclease-resistant phosphorothioate backbone and have been previously used for both parenteral (19) and mucosal immunizations (23). Recombinant glycoprotein B of HSV-1 (Chiron, Emeryville, CA) was used at 10 μg/immunization.

Immunizations

All groups of mice were immunized i.n. with vaccine plus PBS in a total volume of 15 μl, as previously described (34). In short, each mouse was halothane anesthetized and held inverted with nose down until droplets of vaccine that were applied to the external nares were completely inhaled. All mice were immunized twice with a 2-wk interval between.

Collection of fluids and estrous staging

Serum was collected from each mouse 2 wk following a second immunization. Vaginal washes for estrous staging and Ab determinations were collected daily by pipetting 30 μl of PBS into and out of the vagina six to eight times as previously described (33, 34). The staging of the estrous cycle (estrus, metestrus, diestrus, or proestrus) for each mouse was based on the number of days following a vaginal plug (33). Briefly, the vagina, cervix, and uterine horns were removed and the uteri were disrupted, AdgB-infected, syngeneic MC57 cells at a stimulator-to-responder ratio of 1:166 in RPMI 1640 medium (10% FCS). Lymphocytes from ILN, which drain the genital tract, were harvested 48–60 h following intravaginal (IVAG) HSV-2 infection and cultured in vitro for 3 days. Spleen or lymph node effector cells were then cultured with target cells in a 51Cr release assay as previously described (32). MC57 (H-2b) or LTA (H-2k) target cells were cultured with gB CTL peptide or infected with HSV-2 for 5 h after culturing with six effector cells. The cytotoxic activity was calculated using the following formula: % specific lysis = (test counts – spontaneous counts)/(max counts – spontaneous counts). For intracellular IFN-γ staining, ILN cells were cultured for 5 h with gB CTL epitope as above in the presence of Golgi plug (PharMingen) to block protein export and thereby intracellular levels of IFN-γ. Cells were then fixed and permeabilized with Perm/Fix solution (PharMingen) and stained with anti-IFN-γ–FITC and anti-CD8–PE before FACS analysis.

IVAG HSV-2 challenge

Three weeks after the second immunization, mice were injected s.c. with 2 mg of progesterone/mouse (Depo-Provera; Upjohn, Don Mills, Ontario, Canada). Five days later mice were anesthetized using halothane, swabbed IVAG with a cotton applicator, placed on their backs, and infected IVAG with 10^5 PFU of HSV-2 while being maintained under anesthesia. Mice were IVAG washed daily by pipetting 2 × 30 μl of PBS into and out of the vagina six to eight times. Viral titers in IVAG washes were determined by plaque assay on Vero cell monolayers. Genital pathology was monitored daily following HSV-2 challenge, and scoring was performed blinded. Pathology was scored on a five-point scale: 0, no apparent infection; 1, slight redness of external vagina; 2, redness and swelling of external vagina; 3, severe redness and swelling of external vaginal and surrounding tissue; 4, genital ulceration with severe erythema and edema; and 5, severe genital ulceration extending to surrounding tissue. Mice were sacrificed upon reaching stage 5.

Results

CpG ODN administered i.n. with rgB increased serum and genital tract IgG and IgA levels

The levels of specific anti-gB Abs induced following i.n. immunization with rgB alone or mixed with CpG or control non-CpG ODN were evaluated in serum and genital tract washes. Additionally, mice were immunized with AdgB, which we previously showed induced strong mucosal immunity and protection (2), were evaluated. Fig. 1 shows that only AdgB or CpG ODN plus rgB immunization induced significantly greater levels of serum anti-gB IgA and IgG (p < 0.05) than in mice immunized with rgB alone. Furthermore, control mice immunized i.n. with CpG ODN alone did not generate any detectable specific serum anti-gB IgG or IgA Abs (Fig. 1).

Because HSV-2 is a sexually transmitted virus, Ab levels in the genital tract were also examined. Titers of gB-specific IgA Abs in pooled estrus samples and IgG Abs in pooled diestrus samples were found to be highest on average in the genital tract washes of rgB plus CpG ODN-immunized mice (Fig. 1). IgA but not IgG levels were also significantly higher (p < 0.001) in rgB plus CpG ODN-immunized mice compared with mice immunized with rgB alone, and several animals had IgA titers a log higher than in any other group including AdgB. Control mice immunized with CpG ODN alone did not have any detectable specific IgG or IgA Abs in their genital washes (Fig. 1). These results demonstrate the ability of i.n. administered CpG ODN to act as a mucosal adjuvant for the
induction of specific mucosal Ab responses in distant mucosal tissues.

Effects of the estrous cycle on gB-specific Ab levels
The levels of specific and total Abs in the murine genital tract have been shown to rise and fall with the stages of the estrous cycle (33, 36). Here, the ratios of IgA to IgG Abs over one estrous cycle were highest during estrus and lowest during diestrus (Fig. 2), consistent with our previous results (33). Interestingly, although ratios in all groups of immunized mice displayed this same profile, the ratios were up to a log higher in CpG ODN plus rgB-immunized mice, reflecting the overall higher levels of anti-gB IgA found in these animals. As a result, levels of specific IgA were often higher during diestrus in the rgB plus CpG-immunized mice when compared with estrus levels in mice from other groups.

Specific ASC in the genital tract
Next we addressed whether i.n. immunization with CpG ODN plus rgB induced ASC in the genital tract. Four weeks following immunization, mice were IVAG challenged with HSV-2, and the ASC responses were assessed in genital tissues. As early as 2 days after challenge, the numbers of IgA ASC specific for gB were 3-fold and more than a log higher in rgB plus CpG ODN-immunized mice than in mice immunized with rgB plus non-CpG ODN or rgB alone, respectively (Fig. 3). By 6 days post IVAG challenge, numbers of IgA ASC had increased dramatically (over 1600) in CpG plus rgB-immunized mice, but not in any of the other groups of mice (Fig. 3). In contrast to these responses, at days 2 or 6 post IVAG challenge the IgG ASC responses in CpG ODN plus rgB-immunized mice were no different or lower than those of mice immunized with non-CpG ODN plus rgB or AdgB. Interestingly, although initial numbers of IgG ASC were extremely high, they decreased by over a log from days 2 to 6 in all groups. Thus, i.n. CpG ODN plus rgB immunization preferentially induced and maintained large numbers of gB IgA ASC capable of homing to the genital tract in response to local Ag challenge.

Ab ratios
Ratios of gB-specific IgG subclass Abs in serum were examined as an indication of the phenotype of the immune response induced by CpG ODN plus rgB immunization (Fig. 4). Recombinant gB-specific IgG2a-to-IgG1 Ab ratios were found to be significantly greater \((p \leq 0.001)\) in the CpG ODN plus rgB (3.4 ± 1.9 vs 0.5 ± 0.27 for gB/ODN; 0.19 ± 0.087 for gB alone; and 0.9 ± 0.13 for AdgB)-immunized group than in any other group and always >1. In contrast, with the exception of a single animal, mice receiving rgB alone or rgB plus control non-CpG ODN had ratios below 1. These observations demonstrate that an IgG2a-dominated Ab response was seen in the serum of mice immunized i.n. with AdgB and especially in mice immunized i.n. with CpG ODN plus rgB.

![FIGURE 1. Intranasal CpG ODN plus rgB induced high levels of gB-specific IgA Abs in the genital tract and serum. Mice were immunized twice with a 2-wk break, and serum was collected 2 wk later. For Ab levels in the genital tract, daily IVAG washes were taken for each mouse starting 2 wk post second immunization and pooled according to estrus stage as described in Materials and Methods. Levels of gB-specific Abs are expressed as the geometric mean titer for individual (●) mice as well as the mean ± SEM for each group. Differences between groups were calculated by ANOVA.](http://www.jimmunol.org/)

![FIGURE 2. Daily IVAG washes taken from mice 3 wk after receiving a second immunization were analyzed for gB-specific IgA and IgG Ab levels and estrous stage. The IgA-to-IgG ratios were calculated and are shown for individual mice for each day of one complete estrous cycle. D, Diestrus; P, proestrus; E, estrus; M, metestrus.](http://www.jimmunol.org/)

![FIGURE 3. HSV-2-specific B cells in the genital tract 4 wk following i.n. immunization. Mice immunized twice were challenged IVAG with 2 × 10^7 PFU of HSV-2 and 2 or 6 days later lymphocytes were isolated from four pooled genital tracts per group. IgA and IgG gB-specific ASC were identified by ELISPOT.](http://www.jimmunol.org/)
CTLA analysis

The induction of Th1 responses, and predominantly CTL, is considered an integral component of a successful HSV vaccine. In two separate experiments, we examined whether CpG ODN plus rgB-immunized mice contained any CTL against HSV in their spleens. Results from one experiment are shown in Fig. 5, where it is apparent that AdgB immunization resulted in elevated levels of HSV-specific CTL. Together with the second experiment, eight of eight AdgB-immunized mice were found to contain high levels of splenic CTL specific for HSV. In contrast, lower levels of HSV-specific CTL-mediated killing were observed in mice immunized with rgB plus ODN with or without CpG (Fig. 5). Indeed, only three of eight and two of eight mice immunized with either CpG ODN or non-CpG ODN, respectively, had anti-HSV CTL activity in their spleens. HSV-specific CTL were not observed in any animals immunized with rgB alone (Fig. 5) or in control mice immunized with CpG ODN alone (data not shown).

To determine whether week 4 immunized mice contained CTL locally in the genital tract mice were challenged IVAG with HSV-2, and the ILN and genital tract were examined 48–60 h later for CTL specific for HSV-2 as previously described (32). In contrast to splenic CTL activity, high anti-HSV CTL activity was observed in the ILN that drain the genital tract of mice immunized with CpG ODN or non-CpG ODN plus rgB and AdgB (Fig. 6A). Interestingly, only rgB plus CpG and AdgB groups were significantly higher than rgB alone (p ≤ 0.005). When CD8+ T cells (gated for CD8) from the ILN were examined by FACS for IFN-γ production following in vitro gB-peptide stimulation all three groups of mice had significantly higher (p ≤ 0.05) percentages of IFN-γ-positive cells compared with mice immunized with rgB alone (Fig. 6B). In addition to the lymph nodes, the genital tissues were examined for CTL activity by determining the number of IFN-γ-positive cells in response to peptide stimulation by ELISPOT (Fig. 6C) and intracellular IFN-γ staining (data not shown). From these experiments it was clear that mice immunized with AdgB or rgB plus CpG/ODN or rgB plus non-CpG ODN developed CTL rapidly in the genital tissues following IVAG infection.

Protection from IVAG HSV-2 challenge

To evaluate the level of protection mediated by i.n. immunization with CpG ODN plus rgB, mice were challenged IVAG with HSV-2. From both low and high dose challenge experiments it was clear that only mice immunized with CpG ODN plus rgB or AdgB were protected from overt signs of disease (Table I). In addition, overall severity scores were significantly lower in these two groups of mice than those for any of the other groups (Table I; p ≤ 0.05). Actual survival from low dose challenge was also predominantly

FIGURE 5. Moderate levels of HSV-2-specific CTL in the spleen of CpG-immunized mice. Splenocytes from mice immunized twice were isolated and stimulated in vitro for 5 days with Ag. The presence of CTL effectors expanded in vitro was assessed in a 51Cr assay against syngeneic targets infected with HSV-2. Each point represents the percent specific lysis of targets by individual animals at the indicated E:T ratio.

FIGURE 6. High levels of local CTL in the genital tract and lymph nodes of CpG-immunized mice. Immunized mice were challenged IVAG with 2 × 10^7 PFU of HSV-2 and 48–60 h later lymphocytes were isolated from individual ILN or four pooled genital tracts per group. Following 3 days of in vitro culture, the presence of CTL effectors in ILN was evaluated in a 51Cr release assay (A) and by intracellular staining for IFN-γ following gB-peptide stimulation (B). C, Genital tracts from these mice were pooled and digested, and the isolated lymphocytes producing IFN-γ in response to gB-peptide stimulation were enumerated in an IFN-γ ELISPOT assay.
observed in rgB plus CpG ODN- and AdgB-immunized mice. However, high dose challenge overcame this protection, especially in AdgB-immunized mice.

Interestingly, although mice immunized with rgB plus non-CpG ODN induced relatively high levels of anti-gB IgG and IgA and comparable levels of anti-HSV CTL seen in CpG ODN-immunized mice, mice immunized with rgB plus non-CpG ODN were not protected against IVAG challenge with HSV-2. Furthermore, mice immunized with CpG ODN alone were not protected (Table 1 and Fig. 7). These results suggest that mucosal immunization with rgB plus CpG ODN but not non-CpG ODN induced an immune response that is critical for protection from genital HSV-2 challenge. In addition, over the first 2 days after IVAG challenge the levels of virus recovered from mice immunized with rgB plus CpG ODN were up to a log lower than in other groups of mice including those immunized with AdgB (Fig. 7). The ability of rgB plus CpG ODN to achieve such a significant reduction in viral titer is a major achievement with regard to control of sexually transmitted virus infections.

Discussion

In recent years, a large number of studies have demonstrated the immunostimulatory properties of CpG ODN. Although most vaccines using CpG ODN as adjuvant have been administered parenterally, there have been several reports concerning the enhancement of mucosal immune responses following i.n. delivery (23–26). However, no studies have compared the ability of CpG and non-CpG ODN to induce mucosal immune responses in the genital tract and protection from IVAG virus challenge. The results presented here demonstrate that i.n. immunization with CpG ODN plus recombinant gB protein induces strong cellular and humoral immune responses in the genital tract. These responses were characterized by high specific IgA and IgG levels, local specific ASC and CTL responses, and protection from IVAG HSV-2 infection.

Intranasal administration of CpG ODN plus rgB induced high levels of specific IgG and IgA in the serum and genital tract that were significantly greater than mice immunized with rgB alone. Furthermore, i.n. immunization with CpG ODN alone did not induce detectable gB-specific Abs in serum or genital washes. We previously demonstrated that the levels of specific and total Abs in the murine genital tract rise and fall with the stages of the estrous cycle (33). These observations have been confirmed by others (36) and indicate that to properly assess local Ab levels, daily IVAG washes need to be taken from individual mice and the stage of the estrous cycle determined. Following this protocol, and in agreement with our previous findings, the ratios of Ag-specific IgA to IgG Abs were highest during estrus and lowest during diestrus. Interestingly, although all groups of immunized mice displayed this profile, the ratios were up to a log higher in CpG ODN plus rgB-immunized mice, reflecting the overall higher levels of anti-HSVgB IgA in the genital tract of these animals. Importantly, the levels of specific IgA were often higher during diestrus in the rgB plus CpG ODN-immunized mice when compared with estrus levels in mice from other groups. These results indicate that use of CpG ODN as a mucosal adjuvant may induce sufficient levels of specific IgA in the genital tract to protect from infection throughout the murine reproductive cycle. Because similar fluctuations in Ab levels have been associated with the menstrual cycle (37–39), these results may also extend to primates and humans.

To evaluate whether the dramatically elevated levels of IgA in the genital washes of CpG ODN-immunized mice were associated with a local specific B cell immune response, the number of HSVgB-specific ASC in the genital tract were determined. As early as 2 days following IVAG challenge with HSV-2, IgA ASC were observed in the genital tracts of all immunized mice. The numbers of IgA ASC, though, were 3-fold and more than a log higher in rgB plus CpG ODN-immunized mice than in mice immunized with rgB plus non-CpG ODN or rgB alone, respectively. Interestingly, 2 days after IVAG challenge, mice immunized with AdgB had comparable numbers of IgA ASC in the genital tract as CpG ODN-immunized mice but by 6 days after challenge the numbers of IgA ASC had increased dramatically (over 1600) in the CpG-immunized mice but not in any other group. Thus, IgA ASC dramatically increased in number over time following local viral challenge in rgB plus CpG ODN-immunized mice. The high and

Table 1. Protection against HSV-2 in the genital tracts of i.n. immunized mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Low Challenge Dose</th>
<th>High Challenge Dose</th>
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<tbody>
<tr>
<td></td>
<td>Severity</td>
<td>Protection</td>
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<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>gB/CpG</td>
<td>6.1 (3.9)**</td>
<td>2/5</td>
</tr>
<tr>
<td>gB/ODN</td>
<td>20.4 (1.6)</td>
<td>0/5</td>
</tr>
<tr>
<td>gB</td>
<td>19.6 (3.3)</td>
<td>0/5</td>
</tr>
<tr>
<td>AdgB</td>
<td>4.8 (2.5)**</td>
<td>4/6</td>
</tr>
<tr>
<td>PBS</td>
<td>22.7 (2.6)</td>
<td>0/8</td>
</tr>
<tr>
<td>CpG</td>
<td>ND</td>
<td>ND</td>
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<tr>
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<td></td>
<td>Severity</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>gB/CpG</td>
<td>5.8 (1.0)**</td>
<td>2/6</td>
</tr>
<tr>
<td>gB/ODN</td>
<td>10.7 (1.6)</td>
<td>0/6</td>
</tr>
<tr>
<td>gB</td>
<td>13.6 (1.4)</td>
<td>0/6</td>
</tr>
<tr>
<td>AdgB</td>
<td>8.9 (1.7)*</td>
<td>1/6</td>
</tr>
<tr>
<td>PBS</td>
<td>14.1 (1.4)</td>
<td>0/7</td>
</tr>
<tr>
<td>CpG</td>
<td>13.5 (0.9)</td>
<td>0/6</td>
</tr>
</tbody>
</table>

* Mice were IVAG challenged with 2 × 10⁵ PFU (low dose) or 2 × 10⁶ (high dose) of HSV-2.

** Mean ± SEM, measured as area under lesion score-day curve for first 10 days.

** Number of mice that demonstrated no overt genital pathology/total (i.e., pathology score <1).

** Number of mice surviving infection/total (i.e., pathology score <3).

* Mean ± SEM, measured as area under lesion score-day curve for first 6 days.

Significance between gB/CpG or AdgB- and PBS/CpG-treated mice. *p < 0.05; **p < 0.001.

FIGURE 7. Reduced viral shedding in the genital tracts of rgB plus CpG ODN-immunized mice. Mice immunized twice were challenged IVAG with 2 × 10⁵ PFU of HSV-2. Daily IVAG washes were taken, and viral titers were determined on Vero monolayers. The means ± SEM of three mice per group are shown.
sustained number of specific IgA ASC in the genital tract following infection strongly suggests a local component to the elevated levels of IgA observed in genital washes throughout the reproductive cycle. The sustained IgA ASC response may be due to the ability of CpG ODN to directly activate B cells, macrophages, and dendritic cells (11, 12, 14) to secrete Th1-like cytokines such as IFN-γ and IL-12, express costimulatory molecules, and increase Ag presentation (14, 15, 18, 19). In addition, B cell activation by CpG shows strong synergy with signaling through the specific B cell receptor (21) and promotes anti-apoptotic activities (12). Thus, the significant increase and maintenance of Ag-specific IgA ASC following recombinant viral protein plus CpG ODN immunization may reflect the protection of activated B cells from apoptosis or, alternatively, a cytokine/chemokine milieu that induces a population of B cells that can participate in local mucosal immune responses to infection.

Our results also confirm that i.n. immunization with CpG ODN plus rgB, but not non-CpG ODN, induced an IgG2a-dominant Ab response in serum. HSVgB-specific IgG2a-to-IgG1 Ab ratios were significantly greater in the CpG ODN plus rgB-immunized group than in any other group. Similarly, an IgG2a-dominated response was seen in AdgB-immunized mice. IgG2a-dominant responses are typically associated with a Th1 T cell response, and CpG ODN has been shown to predominantly induce Th1 cytokines (20–22). Studies concerned with the induction of anti-HSV CTL demonstrated that although AdgB induced high levels of specific CTL in the spleens of all immunized mice, CpG ODN plus rgB induced moderate CTL levels that were undetectable in some animals. Indeed, comparable levels of HSV-specific CTL-mediated killing were observed in mice immunized with rgB plus ODN with or without CpG. In contrast to splenic CTL, CpG ODN induced high levels of CTL in the ILN that drain the genital tract shortly following IVAG HSV-2 infection. Indeed, the high levels of specific CTL were confirmed in studies that examined the numbers of CD8+ T cells in the ILN that contained IFN-γ in response to gB epitope exposure. Previously we demonstrated that mucosal but not systemic immunization with AdgB led to compartmentalization of anti-viral CTL to mucosal-associated lymph nodes over time (32). Although it has been shown that systemic administration of CpG ODN induces potent CTL responses in the spleen, it seems clear from our results that mucosal administration of CpG ODN promotes a more localized and compartmentalized CTL response in the genital tract.

Following both low and high dose IVAG challenge with HSV-2, only mice immunized with CpG ODN plus rgB or AdgB were protected from overt signs of disease, had significantly lower severity scores, and survived low dose virus challenge. Interestingly, mice immunized with rgB plus non-CpG ODN induced relatively high levels of anti-gB IgG and IgA and comparable levels of anti-HSV CTL in the spleen and genital tract as CpG ODN-immunized mice but were not protected against IVAG challenge with HSV-2. These results suggest that mucosal immunization with rgB plus CpG ODN but not non-CpG ODN induced an immune response that is critical for protection from genital challenge. This could also relate to the observation that non-CpG ODN did not induce an IgG2a-dominant response and thus may not have induced a Th1-like response. In addition, over the first 2 days after IVAG challenge the levels of virus recovered from mice immunized with rgB plus CpG ODN were up to a log lower than in other groups of mice, including those immunized with AdgB. The ability of recombinant viral protein plus CpG ODN to achieve such a significant reduction in the titer of virus in the genital tract is a major achievement with regard to control of sexually transmitted virus infections. The lower levels of free virus in the genital tracts of rgB plus CpG ODN-immunized mice may reflect neutralization of the initial infectious load. Alternatively, virus levels may have appeared lower due to Abs in the genital tract that could have neutralized virus being shed into IVAG washes. Nevertheless, these results are the first to show that mucosal (i.n.) immunization with CpG ODN plus Ag induced significant specific IgA and anti-HSV CTL in the genital tract that remained high throughout the estrous cycle and protected mice from challenge in the genital tract. The sustained presence of specific IgA Abs in the genital tract and the induction of localized HSV-specific CTL may also serve to reduce the transmission of virus from infected to uninfected individuals via sexual transmission.

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